

U.S. Ser. No. 10/511,711
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AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A sandwich assay method for detecting a presence of a target molecule in a sample comprising a complex biological fluid, which assay comprises:

providing a first affinity ligand with affinity for the target molecule, which affinity ligand is capable of being immobilized to a solid support;

applying a sample comprising a complex biological fluid in such a way that binding of a target molecule, if present in the sample comprising a complex biological fluid, to the first affinity ligand is enabled;

applying a second affinity ligand with affinity for the target molecule, the application enabling binding of the second affinity ligand to the target molecule;

removing second affinity ligand not bound to target molecule; and

detecting a presence of the second affinity ligand, such presence being an indicator of the presence of a target molecule in the sample;

the first affinity ligand being immobilized to the solid support at any stage before said detection,

wherein at least one of the first and second affinity ligands is a naturally occurring bacterial reeoptin, a domain thereof, or an engineered protein, constructed from a scaffold

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domain selected from domains of bacterial receptors,
fibronectins, protease inhibitors, retinol binding proteins,
bilin binding proteins, amylase inhibitors, CTLA-4, cytochromes,
or cellulose binding proteins, and further wherein at least one
of the first and second affinity ligands is an affinity ligand
other than an antibody.

2. (Previously Presented) The sandwich assay method according to claim 1, in which the first affinity ligand is provided immobilized to the solid support.

3. (Previously Presented) The sandwich assay method according to claim 1, in which the first affinity ligand is immobilized to the solid support during performance of the method.

4. (Currently Amended) The sandwich assay method according to claim 2, in which the solid support is selected from microtiter plates, plates, compact discs comprising microfluidic channels, channels, protein array chips, chips, membranes, membranes, microparticles, microparticles, pin structures, structures, stick structures, structures, sensor surfaces, surfaces, or cell surfaces.

5. (Previously Presented) The sandwich assay method according to claim 4, in which the solid support is a microtiter plate.

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6. (Previously Presented) The sandwich assay method according to claim 1, which further comprises removing target molecules not bound to the first affinity ligand.

7. (Previously Presented) The sandwich assay method according to claim 1, in which the second affinity ligand is an affinity ligand other than an antibody.

8. (Previously Presented) The sandwich assay method according to claim 1, in which the first affinity ligand is an affinity ligand other than an antibody.

9. (Previously Presented) The sandwich assay method according to claim 1, in which both the first and the second affinity ligand is an affinity ligand other than an antibody.

Claims 10. - 14. (Canceled)

15. (Currently Amended) The sandwich assay method according to claim 1 ~~claim 14~~, in which the scaffold is selected from bacterial receptor domains.

16. (Previously Presented) The sandwich assay method according to claim 15, in which the scaffold is selected from the immunoglobulin binding domains of staphylococcal protein A.

17. (Previously Presented) The sandwich assay method according to claim 16, in which the scaffold is a B domain of staphylococcal protein A.

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18. (Previously Presented) The sandwich assay method according to claim 16, in which the scaffold is a Z domain derived from the B domain of staphylococcal protein A.

19. (Currently Amended) The sandwich assay method according to claim 15 ~~claim 14~~, in which the scaffold is selected from immunoglobulin binding domains of *Peptostreptococcus magnus* protein L.

20. (Currently Amended) The sandwich assay method according to claim 15 ~~claim 14~~, in which the scaffold is selected from immunoglobulin binding domains of streptococcal protein G.

21. (Currently Amended) The sandwich assay method according to claim 15 ~~claim 14~~, in which the scaffold is selected from albumin binding domains of streptococcal protein G.

22. (Currently Amended) The sandwich assay method according to claim 1 ~~claim 14~~, in which the engineered protein ~~used as affinity ligand~~ is selected from a library of variants of the selected scaffold used.

23. (Previously Presented) The sandwich assay method according to claim 22, in which the library is a combinatorial library.

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24. (Currently Amended) The sandwich assay method according to claim 22, in which the library is constructed with using phage display technology.

25. (Currently Amended) The sandwich assay method according to claim 1, in which the engineered protein at least one of the first and second affinity ligands is derived from a library of linear peptides.

26. (Currently Amended) The sandwich assay method according to claim 1, in which the engineered protein at least one of the first and second affinity ligands is derived from a library of cyclic peptides.

Claims 27. - 31. (Canceled).

32. (Previously Presented) The sandwich assay method according to claim 1, in which the complex biological fluid is selected from serum, plasma, saliva, whole blood, plasma from plasmapheresis, cerebrospinal fluid, amniotic fluid, urine, semen, cord blood, supernatants from cell culture, cell culture media, exsudate or aspirate.

33. (Previously Presented) The sandwich assay method according to claim 1, in which the sample is a human sample.

34. (Previously Presented) The sandwich assay method according to claim 33, in which the sample is a human serum sample.

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35. (Currently Amended) A kit for use in a sandwich assay method, the kit comprising:

a first affinity ligand with affinity for a target molecule and capable of being immobilized to a solid support;

a second affinity ligand with affinity for the target molecule, a presence of which ligand is detectable; and

a solid support to which the first affinity ligand is capable of being immobilized,

wherein at least one of the first and second affinity ligands is a naturally occurring bacterial receptor, a domain thereof, or an engineered protein, constructed from a scaffold domain selected from domains of bacterial receptors, fibronectins, protease inhibitors, retinol binding proteins, bilin binding proteins, amylase inhibitors, CTLA-4, cytochromes, or cellulose binding proteins and further wherein at least one of the first and second affinity ligands is an affinity ligand other than an antibody.

36. (Currently Amended) The kit according to claim 35, wherein the solid support is selected from the group consisting of microtiter plates, plates, compact discs comprising microfluidic channels, channels, protein array chips, chips, membranes, membranes, microparticles, microparticles, pin structures, structures, stick structures, structures, sensor surfaces, surfaces, and cell surfaces.

Claims 37.-40. (Canceled)

41. (Currently Amended) The sandwich assay method according to claim 3, in which the solid support is selected

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from microtiter plates, plates, compact discs comprising microfluidic channels, channels, protein array chips, chips, membranes, membranes, microparticles, microparticles, pin structures, structures, stick structures, structures, sensor surfaces, surfaces, or cell surfaces.

42. (Previously Presented) The sandwich assay method according to claim 41, in which the solid support is a microtiter plate.